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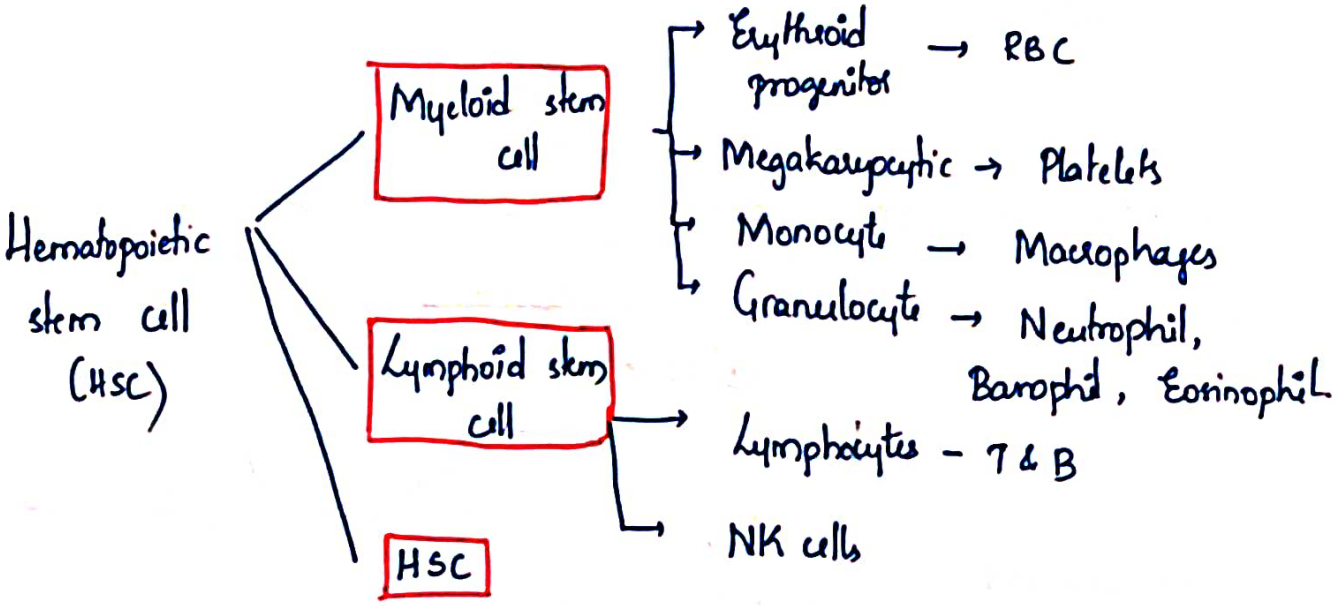
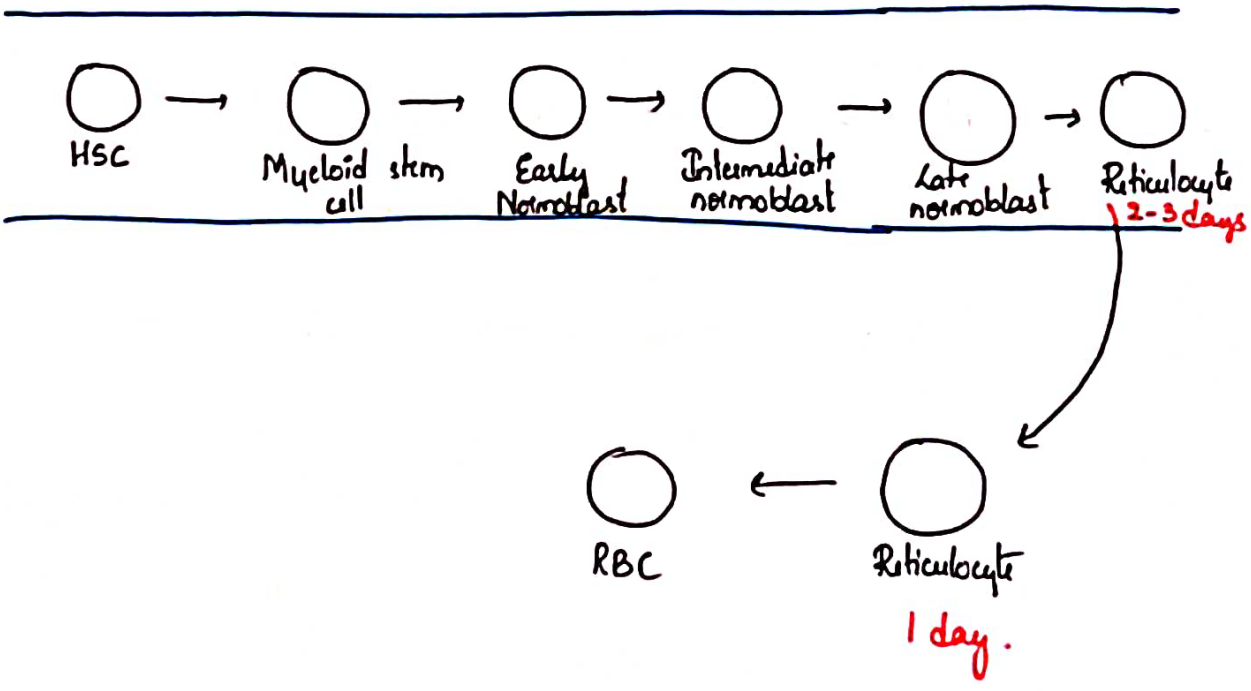
HEMATOLOGY

ANEMIA INTRODUCTION





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Reticulocyte

Ex : Hb = 5gm %
Retic % = 6 %

Corrected Reticulocyte Index = Retic % × $\frac{Hb}{\text{(N) Hb}}$ = Retic % × $\frac{Hct}{\text{(N) Hct.}}$

(N) Hb → M = 15
F = 14

Hb × 3 = Hct (Hematocrit)

∴ Corrected retic index = 6 % × $\frac{5}{15}$ = 2 %

Hb = 15 ⟶ (N) maturation time is 1 day

Hb = 8-10 — 2 days - Maturation time

Hb = 5 — 3 days - "

Retic production index = $\frac{\text{Corrected retic index}}{\text{Maturation (correction factor)}}$

∴ Retic production index = $\frac{2}{3}$ = < 1 %.

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In most cases,

Retic production index = $\frac{\text{Corrected retic \%}}{2}$

Anemia pt

↓

Retic index.

↓

High

↓

Bleeding → Yes → Blood loss

↓

No

↓

Peripheral blood smear

- Schistocytes – MAHA
- Spherocytes – • Warm autoimmune hemolytic anemia
  • Hereditary spherocytosis
- Elliptocytes – H. elliptocytosis
- Sickle cells – Sickle cell disease
- Bite cells – G-6-PD def
- Target cell – Thalassemia
- Inclusions – Malaria

Low

↓

Peripheral blood smear

↓

Microcytic

↓

- Iron def
- Thalassemia

Normocytic

↓

- Anemia of chr ds
- CKD

Macrocytic

↓

- B_{12} def
- Folate def
- Myelodysplasia
- Alcohol
- Drug toxicity.

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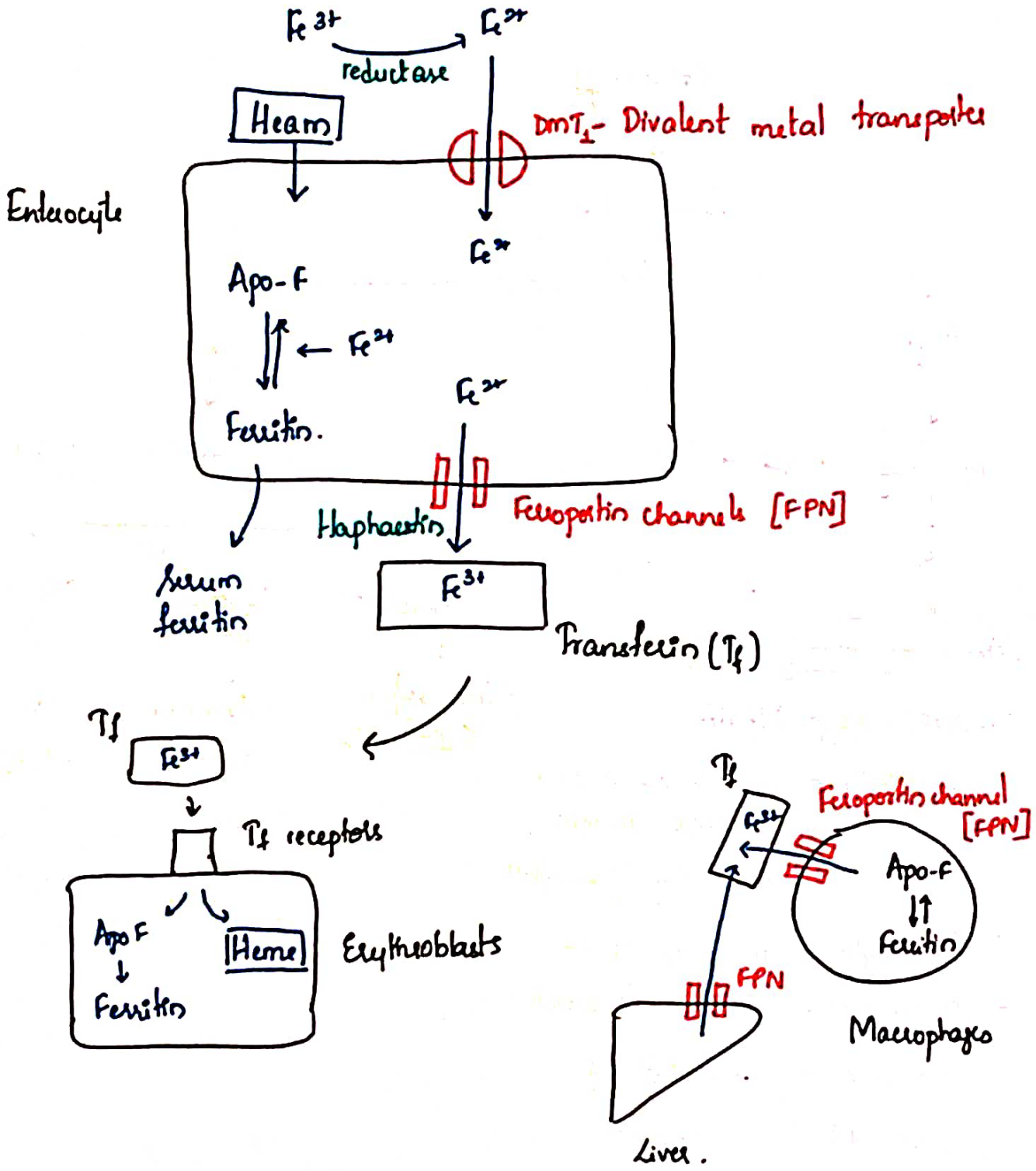
IRON HOMEOSTASIS

Absorption : Duodenum.



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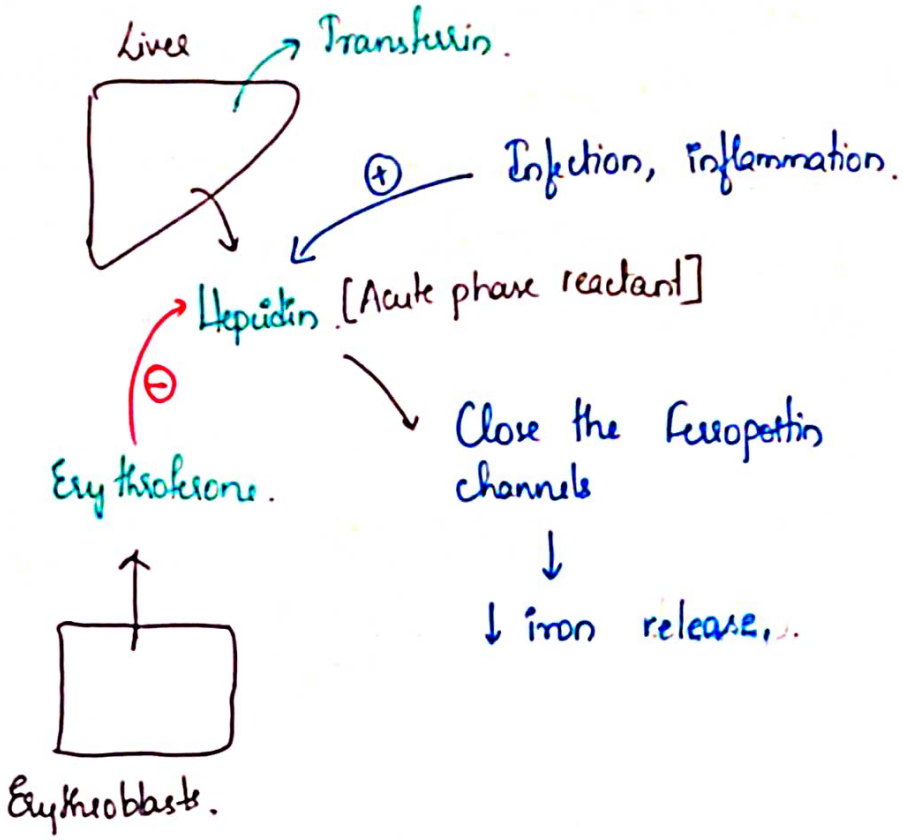

- Total iron binding capacity (TIBC) → ↑ when ferritin ↓
  ↓ when ferritin ↑.

- Iron def → Stores ↓ first then Serum Fe ↓ → Ferritin ↓ (Specific)
  - Soluble transferrin receptor ↑ (Sensitive)

- Chronic inflammation → ↑ Hepcidin → locks up store
  ↓
  Serum Fe ↓
  but Normal stores.
  ↓
  As ferritin ↑
  ∴ TIBC ↓
  bcz Tf Receptor - N

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Hereditary Hemochromatosis → mutation in HFE gene. [needed for production of hepcidin] → ↓ Hepcidin → ↑ Fe absorption & release.

- T_f saturation ↑
- Free radicals → Organ damage. [by Fenton reaction]

Iron content (mg)

	Adult male	Adult female
Hemoglobin	2500	1700
Myoglobin / enzymes	500	300
Transferrin iron	3	3
Iron stores	600 - 1000	0 - 300

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Hepcidin regulators

- Iron regulator → ↑ Tf saturation → ↑ Hepcidin
- Erythroid regulator → Erythroferon → ↓ Hepcidin
  [Proliferating marrow]
- Inflammatory regulator → ↑ IL-6 → ↑ Hepcidin
- Hypoxia regulator → Hypoxia → ↓ Hepcidin

∘ Very high elemental iron → ↑ Hepicidin → ↓ Fe absorption
[3 times a day
$FeSO_4$]

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Microcytic Anemias

Normal values :

S. iron = 50 - 150 ug/dL

S. Ferritin = 30 - 300 ug/L

TIBC = 300 - 400 ug/dL
[250 - 450 ug/dl]

Transferrin saturation = 20 - 50% (33%)

MCV = 80 - 100 fl - femto = 10^{-15}

RDW = 11 - 15

Transferrin saturation = $\frac{\text{S. iron}}{\text{TIBC}} \times 100$

RDW - Red cell distribution width. [CoV]

Intra group variation → Coefficient of variation [CoV] = $\frac{SD}{Mean} \times 100$

RDW - Variation in size [Anisocytosis]

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Raw material deficiency → fluctuation of raw material → varying RBC size → ↑RDW

- Fe def
- B_{12} def
- Folate def

MCV

Microcytic [<80 fl]	Normocytic [80-100 fl]	Macrocytic [>100 fl]

- Iron def
- Thalassemia
- Anemia of chronic disease
- Lead poisoning
- Sideroblastic anemia
- Hbf trait.
	- Anemia of chronic ds
	  - Anemia of che inflammatio
	  - Anemia of CKD
	- Acute blood loss
	- Sickle cell anemia
		- Megaloblastic anemia
		  - B_{12} def
		  - Folate def.
		- Reticulocytosis
		- Alcoholic liver disease
		- Hypothyroid
		- Myelodysplasia

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Iron def anemia

Low input:

- Malabsorption
- Inadequate diet - developing countries.
- Gastric bypass

High output def:

- Bleeding - m.imp in developed countries
- Chronic hemolysis

↑ demand:

- Rapid growth in infancy / adolescence
- Pregnancy
- Erythropoietin therapy

CF:

- Fatigue / Lethargy
- Epithelial changes
- Inadequate muscle function
- ↓ immune competence
- Growth ab(N)
- Cardiac failure. → High output cardiac failure
- Gastritis
- Restless leg syndrome **++**
- Neuropsychiatric

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Epithelial changes :

Nails - Flattening
Koilonychia

Tongue - Soreness
Papillary atrophy -
Filiform papillae - absent/↓

Mouth - Angular stomatitis

Hypopharynx - Dysphagia
Esophageal web } + Iron def anemia

↓

- Plummer Vinson Syndrome
- Patterson Kelly "

Stomach - Achlorhydria
- Gastritis

Lab :

Iron studies : S. ferritin - ↓
TIBC - ↑
S iron - ↓
} Latent iron def anemia
↳ Normocytic / Normochromic anemia.

↓

Late : Microcytic hypochromic anemia.

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Ans :

S. ferritin (↓) - Highly specific, in (↓).

Soluble transferrin receptor - (↑) - High sensitivity.

Transferrin ferritin index

$$= \frac{\text{Sol Tf Receptor}}{\log \text{ferritin}}$$: > 2 → IDA
< 1 → Anemia of chronic dx.

RDW > 15. · If RDW < 15 → Thal. minor.

Mentzer's index = $\frac{\text{MCV}}{\text{RBC (in million)}}$

(N) = 13 - 14.

< 13 = Thalassemia

> 14 = IDA.

- Fecal occult blood testing [FOBT/FIT]
  - ↳ important in developed countries
  - ↳ (+) → Colonoscopy
- Fecal ova / worms in developing countries

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- Celiac → Serology ⊕

Peripheral blood smear: → Image : 31:50

- Microcytic hypochromic
- Platelets ⊕ - * Thrombocytosis ⊕

Nucleus of lymphocytes → reference
└ compared with size of RBC

Hypochromia → enlargement of central pallor.

℞:

Measure Baseline serum iron

1hr ↓ Give 325mg $FeSO_4$ - oral

S. Iron should ↑ 100 ug/dl → Oral Rx.
└ If not - go for parenteral

- Acidic pH → ↑ Fe absp.

(Ascorbic acid)
+
$FeSO_4$

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Elemental iron

Oral → Ferrous SO_4 - 65 mg

Ferrous fumarate - 105 mg

Ferrous gluconate - 28 - 36 mg

(Max) rate of PO iron absp → 25 mg/day.

[∵ large doses - worst help]

(Max) rate of Hb regeneration → 1g% per 3-4 weeks

IV - Iron dextran

		SFG (Sodium ferric gluconate)
Max dose	500 - 1000 mg	125 mg
Test dose	Required	Not required
Administration time	2-4 hours	10 min
Utilization time	weeks	days
Anaphylatic reactions	uncommon	very rare
Delayed reactions	common	rare

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Other new :

- ferumoxytol
- iron-sucrose
- LMW iron dextran
- ferric carboxymaltose

Formula :

Parenteral iron = Body wt × 2.3 × (15 - Hb) + 500/1000mg (for stores)

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ANEMIA - 4

Anemia of chronic disease:

↓

Chronic inflammation / infection

- Hb = 7-12 g/dl. [mild / moderate - never severe]
- Cause:
  - Infection - HIV, TB, Malaria, Osteomyelitis, Chr abscesses, Sepsis
  - Inflammation - Rheumatoid arthritis, IBD, SIRS
  - Malignancy - Carcinoma, Multiple myeloma, lymphoma
  - Cytokine dysregulation - Anemia of aging.
- PBS - Normocytic / Normochromic
  Microcytic / Hypochromic

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Patho :

- ↑IL-6* → ↑ Hepcidin → ↓ Ferroportin channels

⇓

- Ferritin ↑ → TIBC ↓/N
- S. iron - ↓
- Sol Tf receptor - N
- Transferrin ferritin index < 1
  - Sol Tf R / log ferritin.

- ↑ erythrocyte clearance by spleen → ↓ life span.
- ↓ Erythroid colony forming
  ↓ Erythropoietin } IL-1, TNF, INF.
- Erythropoietin ↓ / Erythropoietin resistance.

Iron profile :

S iron - ↓
S. ferritin - ↑
TIBC - ↓/N
Transferrin ferritin index - < 1.

↑ESR, ↑CRP

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Anemia of CKD

- ↓ Epo secretion [Erythropoietin]

Rx: Erythropoietin.

Pre-requisite: Ferritin >500ug/ml
Tf saturation >30%.
} Iron stores

Sideroblastic anemia:

- Idiopathic
- Congenital - δ ALA def (XLR)
- Acquired - Alcohol - (M.C) cause overall.
  - Lead, Zn toxicities
  - Pyridoxine, Cu def.

Patho: Heme formation is defective.
↓
Fe accumulates in cell.

- Ineffective hematopoiesis

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Cfs:

- Pallor
- Dizziness / fatigue
- Liver disease - Hepatomegaly
- Cardiac dysfunction - Arrythmias
- Renal dysfunction

PBS: Image 24:55

- Pappenheimer bodies
- Lead → Basophilic stippling —
- Congenital - MCV ↓ - Microcytosis
- Acq - MCV (N) / ↑ - Macrocytosis

Lab:

- Hb (↓)
- MCV - (↓) / (↑)
- Serum iron - (↑)
- Ferritin - (↑)
- TIBC - (↓) / (N)
- Tf saturation - (↑) / (N).

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Bone marrow biopsy:

- Erythroid hyperplasia → maturation arrest

  > 40% – Ringed sideroblasts

  [Prussian blue stain]

Rx:

- Correct underlying cause.
- Iron chelators.

	IDA	Thal trait	Anemia of chr inflammation
S. iron	↓	N	↓
S. ferritin	↓	N	↑
TIBC	↑	N	↓ / ~
Tf saturation	↓	N	↓ / ~
TFI =	>2	N (1-2)	<1
MCV	↓	↓↓↓ Disproportionate microcytosis	~ / ↓.

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	IDA	Thal minor	Anemia of Chr ds
RBC count -	< 5 million/cumm	> 5 million/cumm	N / low
Mentzer's index = MCV / RBC	> 13	< 13.	N / ↑
RDW	> 15	< 15	

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MACROCYTIC ANEMIAS

MCV > 100 fl

Megaloblastic

Nuclear - cytoplasmic asynchrony (+)

- Vit B_{12} def
- Folate def

Drugs:

- Metformin - ↓ B_{12}
- Methotrexate } Non-megaloblastic
- Zidovudine }

Non-megaloblastic

- Alcohol
- Liver disease
- Myelodysplasia
- Hypothyroid

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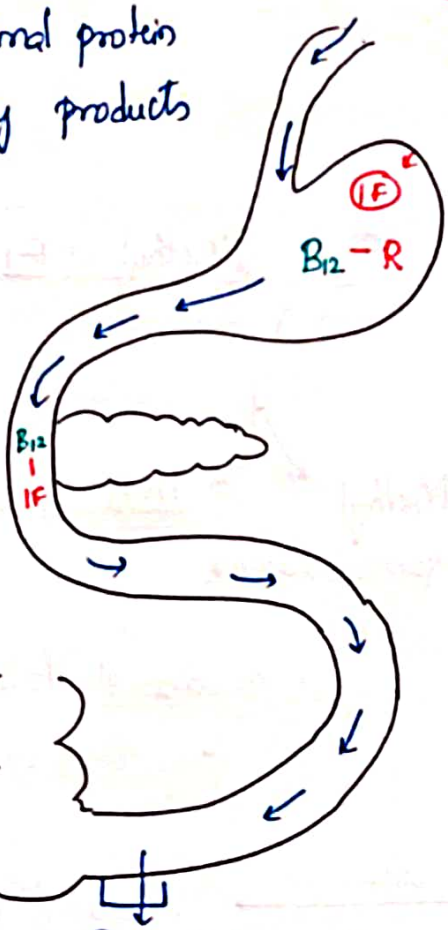
Vit B_{12} def

Protein - B_{12} (R) → Haptocorrin by saliva.

Intake - Animal protein
Dairy products



HCl → Pepsinogen → Pepsin.
↓
digest protein & releases B_{12}

Proteases from pancreas break R factor.

Transcobalamin-II
in terminal ileum

Causes:

- ↓ intake - Vegetarian
- ↓ HCl - Chronic PPI intake, Gastrectomy.
- Pancreatic dysfunction
- Pernicious anemia → ↓IF secretion.
  Abs to parietal cells.
- Ileal disease - Crohn's, bacterial overgrowth.

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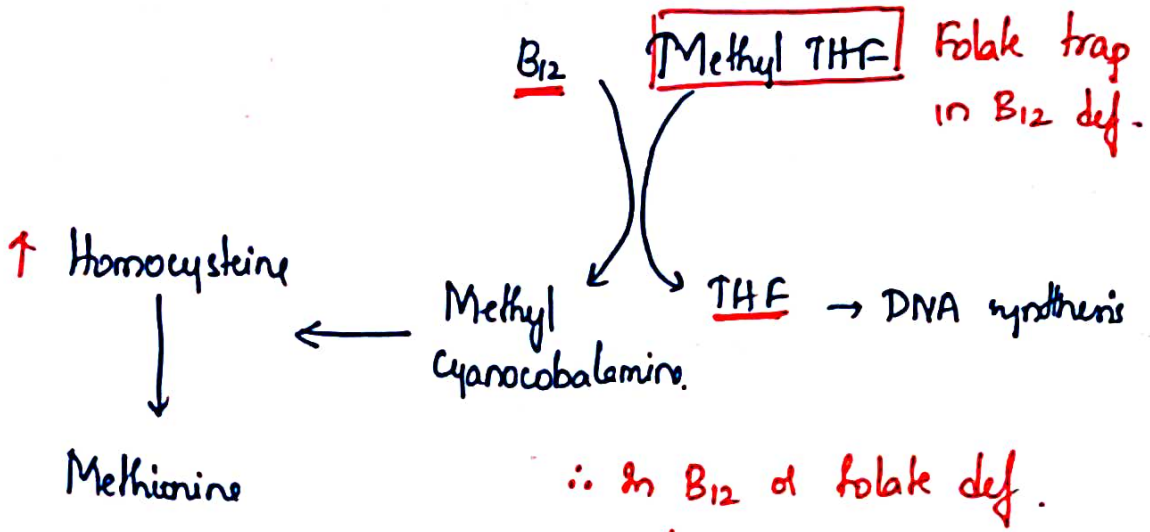
- Diphyllobothrium latum - fish tapeworm.

B_{12} reactions:

1)



2) Methyl malonyl CoA $\xrightarrow{B_{12}.}$ Succinyl CoA → TCA

CFs:

Anemia:
- Fatigue
- Headache
- Pallor
- Dyspnea
- Exercise limitation
- Jaundice - lemon yellow color.

B_{12} def
- Neuro
  - Neuropathy
  - SACD [subacute combined degeneration]

Etiology
- Achlorhydria
- Pancreatitis
- Ileal resection

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Anemia

- Splenomegaly
- Hepatomegaly.
- Retic count ↓.
- Ineffective erythropoiesis

B_{12} def

- Vibration loss (early)
- Dementia

Etiology

- Lactic acidosis [Bac overgrowth]

Pernicious anemia

- Autoimmune gastritis → Tcell in lamina propria
- Neuropsychiatric manifestation precede anemia
- Achlorhydria
  ↓
  No feedback ↓ in Gastrin
  ↓
  ∴ ↑ Gastrin → Hyperplasia
  ↓
  Dysplasia & Carcinoma.

Normal:

↓ Gastrin.
↑
↓
D cells → Somatostatin

- Abs against Parietal cells – (In)
  " " Intrinsic factor – (Sp).
- ↑ risk of gastric Ca

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Lab:

- MCV - ↑
- RDW - ↑
- Anemia → late Pancytopenia
- Low retic count [ineffective erythropoiesis]
- ↑ LDH
- ↑ Bilirubin
- ↓ Haptoglobin
- Fe ↑, ferritin ↑ - excess breakdown of cells.

PBS:

- Hypersegmented neutrophils:
  - ↳ ≥5 lobes in ≥5% neutrophils.

Lab:

- B_{12} [N = 150 - 1000 pg/ml (ng/L)
  - 100 - 200 pg/ml → Check methylmalonic acid. [MMA]
- * MMA - (↑)

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Schilling's test

B_{12} - (I.m) - 1000 ug [fill up stores]

↓

Radioactive B_{12} - (oral)

↓

Urine - B_{12} - radiolabelled - >10% - Normal absorption
< 5% - Absorption problem. [Malabsorption]

↓

* (IF) + Oral B_{12} radiolabelled

↓

Urin B_{12} - (↑) - Pernicious anemia

↓

(↓)

↓

Pancreatic juices + B_{12} - radiolabelled

↓

Urine B_{12} - (↑) - Pancreatitis

↓

(↓)

↓

Antibiotic + B_{12}

↓ → (↑) - Bacterial overgrowth in small intestine.

↳ (↓) - Ileal disease.

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℞ :

Initial - 1000 ug (i.m)

later
- Injection - i.m 1000 ug - every month
- 1000 ug PO daily
- 500 ug intranasal / week.

In neuro affected patients - parenteral B_{12} (i.m)

Folate deficiency

Folate → THF → DNA synthesis

Source : Plant sources
[Green leafy vegetables]

Daily requirement : 150 ug
Stores : 10 - 12 mg (3 - 4 months)

Causes of folate def :
- Poor dietary intake
- ↑ demands - Pregnancy
- Intestinal malabsorption

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- ↓ utilization - Drugs
  Enzyme.
  B_{12} def

- Drugs -

  Methotrexate - Folate antagonist
  Alcohol, OCPs - ↓ absorption
  Sulpha drugs, Cotrimoxazole
  Anticonvulsants - Phenytoin, Phenobarbitone

	B_{12} def	Folate def.
- Anemia -	Megaloblastic	Megaloblastic
Pancytopenia	(+)	(+)
Retic count	↓	↓
Achlorhydria*	+++	(-)
Neurological manifestations*	++	(-)
Homocysteine	↑	↑
Methyl malonic acid	↑↑	(N)
Gastric Ca	(+)	(-)
Abs	(+)	(-)

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B_{12} def	Folate def
Metformin	Phenytoin
Colchicine	OCPs
Neomycin	Alcohol
Chronic PPI	Methotrexate
	Cotrimoxazole

Inv:

- S. folate - <2ng/ml → deficiency
  4-20 ng/ml - @.
- Red cell folate

Rx:

Folic acid - 5- 15 mg/day.

Folinic acid [better especially with drugs like methotrexate]

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HEMOLYTIC ANEMIA

Anemia
↓
Retic index

<2.5

- Iron def
- Anemia of chr ds
- Aplastic anemia
- CKD
- Maligrancy

>2.5

- Hemolysis

Extrinsic

- Abs - Autoimmune hemolytic anemia (AIHA)
- Complement - AIHA
  PNH
- Fragmentation - MAHA
  Metallic valves

Intrinsic

- Membrane
  Hereditary spherocytosis
  Hereditary elliptocytosis
- Enzyme defects
  G6PD def
- Hb defects
  Sickle cell
  Thalassemia

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Non-immune hemolytic

→ **Intrinsic :**

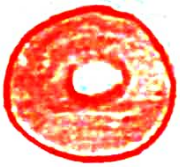
Membrane disorders :

@ RBC

Spherical.

↓ Surface : Volume ratio.

↓



Central pallor.

No central pallor.

Spherocytes

Image: 9:02 9:24.

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Image : 10:04 .

Image : 11:55

Image : 12:42

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Image : 14 : 28

- MCHC ↑
- MCV ↓.

Image : 16 : 19

(M.C) mutation in hereditary spherocytosis : Ankyrin

most severe " " : Spectrin.

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Spherocytes :

- major destruction → Spleen [extravascular hemolysis]

- RBC → macrophages
  ↓
  Hb
  ↙ ↘
  Heam — Globin
  ↙ ↘
  Iron — Protoporphyrin
  ↓ — ↓
  Ferritin [recycled] — Unconjugated bilirubin ↑.
  ↓
  - Jaundice. [Lemon yellow]
  - Gall stones* [Pigmented].

CFs:

- Anemia → Pallor, fatigue, Breathlessness, Heart failure.
- Jaundice
- Gallstones
- Splenomegaly.

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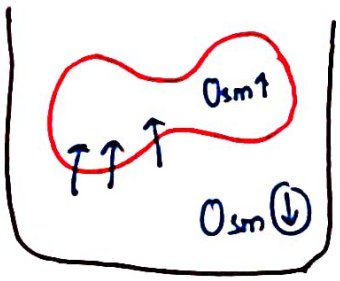
- PBS - Spherocytes.

↓

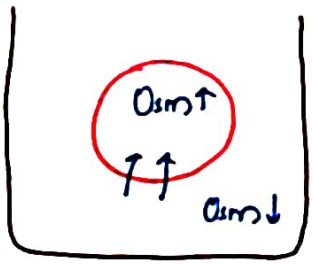
D/D - Hereditary spherocytosis
Autoimmune hemolytic anemia.

- Coomb's test : Negative
- Osmotic fragility - ↑.

RBCs are put in hypotonic solution



Normal RBCs can withstand the hypotonic insult & swell.



Membrane rupture easily

↓

Early lysis of RBC.

↓ osmotic fragility :

- Thalassemia
- Severe iron def anemia
- Hereditary persistence of fetal Hb.

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- Eosin - 5 - maleimide :

↳ attaches to Band - 3.

In HS, ↓attachment due to ankyrin, band 3 defect.

↓

Flow cytometry.

Image : 31 : 28

32 : 24.

Image : 33 : 41

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Lab :

- Hb ↓
- MCV - N / ↓
- MCHC - ↑ [N = 33 g/dl]
- Retic count ↑
- USG - Splenomegaly
  Gall stones
- Unconjugated bilirubin ↑

H. Spherocytosis :

- Autosomal dominant - m.d defect - Ankyrin, Family H ⊕.
- Autosomal recessive

Molecular diagnostics.
Gene testing.

H. elliptocytosis

PBS → Elliptocytes.

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Rx :

- Vaccines of pneumococcal, meningococcal, H.influenza should be given. [Capsulated organisms]
  ↓
  as later splenectomy might be required.

- Splenectomy - Spherocytes will persist.
  ↳ ↑ risk of infections.
  ↑ risk of microinfarcts.

- Severe anemia - Transfusion
  - Erythropoietin.

Image : 43:07

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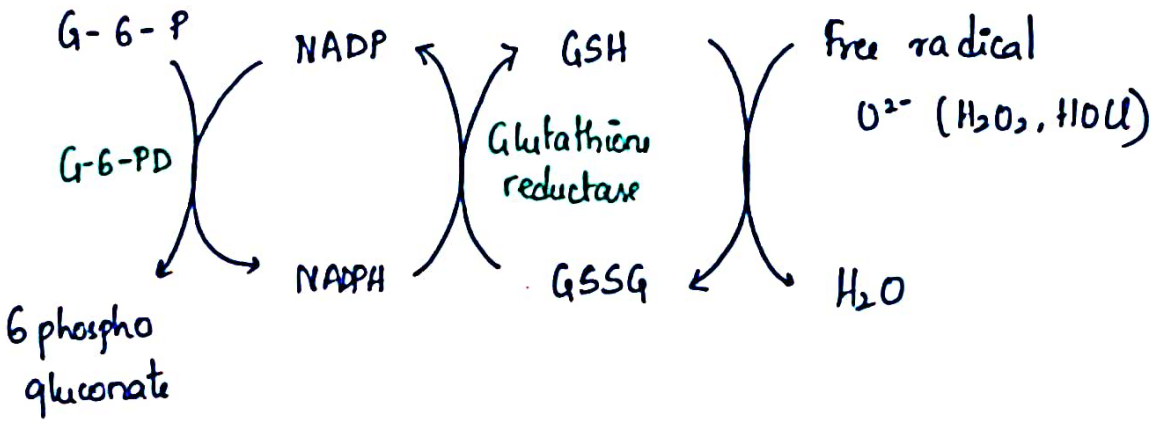
G-6-PD deficiency

Image 45 : 34

Oxidative stress ⟶ Hemolysis [intravascular]



Free radicals : - Fava beans [Favism]
- Drugs (antimalarials)
- Infections

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G-6-PD def
Sickle cell } more common in blacks

↓

Protection against malaria.

Image : 55 : 51

Image : 57 : 25

G6PD

- X linked disease
- Males affected
- Females - carriers
- Females affected if
  - Mother - Carrier
  - Father - Diseased.

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Drugs:

- Antimalarials - Primaquine
  Quinacrine
- Sulpha drugs
- Nitrofurans
- Analgesics - Aspirin
  (rare) Paracetamol
- Dapsone
- Miscellaneous: - Dimercaprol
  Methylene Blue.

CFs:

- Acute hemolytic episodes
- Anemia - Fatigue, breathlessness
- Malaise, weakness, Abdominal / lumbar pain
- Hemoglobinuria, Jaundice.

Lab:

- Haptoglobin ↓. → Intravascular hemolysis

  Hb + Haptoglobin → phagocytosed → Iron is recycled.

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- Hemoglobinuria
- Anemia
- Jaundice.

PBS :

- Bite cells
- Heinz bodies - oxidised denatured Hb clumps in RBCs.
  - seen in supravital stain.

Image : 65 : 16

Image : 65 : 53

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℞:

- Avoid drugs - that triggers hemolysis.
- Vit E, Desferoxamine.

Enzyme:

Gd B - (N)
Gd A - (m.c) variant - G6PD def
Gd Med - lifespan <20 days.

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